

APPLICATION
FOR
UNITED STATES LETTERS PATENT

TITLE: HS-40 ENHANCER-CONTAINING VECTOR
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HS-40 ENHANCER-CONTAINING VECTOR

Background of the Invention

5 HS-40 is a 350-400 bp enhancer element located about
40 kb upstream of ζ -globin gene, which is expressed in the
human embryonic erythroblasts but not in the human adult
erythroblasts. Specific elements within the HS-40 enhancer
have been identified, including GATA-1 motifs, NF-E2/AP1
10 motifs (a 3' and a 5' motif), and a Sp1 binding site.

Summary of the Invention

The invention is based on the discovery that a
single nucleotide change in the 3'NF-E2/AP1 element of the
human HS-40 enhancer, unlike the wild type HS-40 enhancer,
15 confers position-independent and copy number-dependent
expression on a transgene. In addition, the single
nucleotide change allows expression of the gene in the cells
of an adult mouse, an effect not seen for the wild type
HS-40 enhancer.

20 Accordingly, the invention features a viral
expression vector (e.g., a retrovirus) having a nucleic acid
including (1) a transcriptional start site; (2) a promoter
(e.g., a tissue-specific promoter such as a ζ -globin
promoter) operably linked to the transcriptional start site;
25 and (3) an enhancer operably linked to the promoter, the
enhancer including the mutated NF-E2/AP1 (mtNF-E2/AP1) DNA
sequence TCTGAGTCA (SEQ ID NO:1) or the RNA equivalent
thereof. The underlined "T" represents a mutation of the
wild type "G" in the wild type NF-E2/AP1 (wtNF-E2/AP1)
30 sequence. In a specific embodiment, the enhancer includes
the minimal mutated HS-40 DNA sequence

AGATAACTGGGCCAACCATGACTCAGTGCTTCTGGAGGCCAACAGGACT**TCTGAGTCATC**
CTGTGGGGGTGGAGGTGGGACAAGGGAAAGGGGTGAATGGTACTGCTGATTACAACCTCT
GGTGCTGCCTCCCCCTCCTGTTTATCT (SEQ ID NO:2)

or an RNA equivalent thereof. The bold sequence represents
the mtNF-E2/AP1 site with the G to T mutation underlined.
The minimal HS-40 enhancer sequence excludes a 5' GATA-1(b)
site because it has been shown that this site is not
necessary for HS-40 enhancer activity (Zhang et al., J Biol
Chem 270:8501-8505, 1995).

The enhancer can also include the full mutated HS-40
enhancer sequence:

TCGACCCTCTGGAACCTATCAGGGACCACAGTCAGCCAGGCAAGCACATCTGCCCCAAGCC
AAGGGTGGAGGCATGCAGCTGTGGGGGTCTGTGAAAACACTTGAGGGAGCAGATAACTGG
GCCAACCATGACTCAGTGCTTCTGGAGGCCAACAGGACT**TCTGAGTCATC**CCTGTGGGGGT
GGAGGTGGGACAAGGGAAAGGGGTGAATGGTACTGCTGATTACAACCTCTGGTGCTGCCT
CCCCCTCCTGTTTATCTGAGAGGGAAGGCCATGCCCAAAGTGTTTACAGCCAGGCTTCAG
GGGCAAAGCCTGACCCAGACAGTAAATACGTTCTTCATCTGGAGCTGAAGAAATTC
(SEQ ID NO:3)

or an RNA equivalent thereof. The bold sequence represents
the mtnf-E2/AP1 site with the G to T mutation underlined.
This sequence is referred to herein as the mtHS-40 sequence,
which differs from the wild type HS-40 (wtHS-40) sequence by
the G/T mutation indicated above. Again, the single
mutation is underlined. The vector can also contain a
transcriptional termination signal (e.g., a polyadenylation
signal). In other embodiments, the promoter drives
transcription of a mRNA encoding a polypeptide (e.g., a
growth hormone), the transcription beginning from the
transcriptional start site.